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Induction of apoptosis by troglitazone requires peroxisome proliferator-activated receptor gamma and ERK in lung cancer cells

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Troglitazone (TGZ), a synthetic PPAR gamma ligand, is able to induce cell growth arrest and apoptosis in human lung cancer cells, but its pathway is unclear. We therefore studied the role of ERK1/2 in NCI-H23 lung cancer cells treated by TGZ. We found that TGZ induced PPAR gamma and activated ERK1/2 accumulation in the nucleus, where the co-localization of both proteins was found and that the activation of ERK1/2 resulted in apoptosis via the mitochondrial pathway, reflected by the reduction of mitochondria membrane potential, change in Bcl-2 family members, release of cytochrome C into cytosol, and activation of caspase 9. Our study has demonstrated that TGZ induced apoptosis in lung cancer cells via a mitochondrial pathway, and this pathway was PPAR gamma- and ERK-dependent. The interaction between PPAR gamma and ERK may create an auto-regulatory and positive feedback loop to enhance the effect of ERK, whereas the activation of Akt may generate a negative feedback loop to control the degree of apoptosis which occurred in lung cancer cells. (Supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, CUHK 4390/03M).

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Human fetal development is necessary for leukocytic TLR maturation against bacterial infection

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TLR2 and TLR4 mainly respond to bacterial infections dependent upon their receptor and signal protein expression. Higher susceptibility of human neonates to bacterial infection compared to adults may be related to the deficiency of TLR2/4 or its adapter protein on mononuclear leukocytes. To understand this, we studied TLR2/4, MD2, MyD88, IRAK, JUK and NFκB expression using human mononuclear leukocytes (MNC) isolated from first and second trimester fetal liver, umbilical cord blood as well as adolescent peripheral blood. In this study, we compared lipopolysaccharide (LPS)-induced production of α-defensin from MNC and found TLR and MyD88 expression enhanced following the fetal maturation, while IRAK and NFκB expressions remained the same. Most interestingly, MD2 was undetectable in fetal liver MNC but expressed weekly in cord MNC and strongly in adolescent MNC cultures. In addition, only adolescent MNC increases the expression of TLR2/4, IRAK, NFκB and α-defensin after LPS exposure. LPS slightly increases TLR2/4 which express intensity in cord MNC culture, but not in fetal MNC cultures. Taken together, although TLR2/4 and its signals express in all MNC, the effect of LPS stimulation is mainly shown in the adolescent MNC cultures rather than in fetal leukocytes. TLR4 becomes a fully functional receptor against gram negative bacterial invasion dependent on the developmental courses. Lack of the MD2 may be responsible for fetal MNC immunodeficiency against the microbial infection due to failure of TLR4 binding to LPS. The gene controlling of MD2 expression deserves further study.

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Novel function of POSH, a JNK scaffold, as an E3 ubiquitin ligase for the Hrs stability on early endosomes

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POSH (plenty of SH3s) acts as a scaffold that links activated Rac1 and downstream c-Jun N-terminal kinase (JNK) signaling modules. However, it is unknown whether its functional-domain-mediated roles include the interesting RING-finger domain or its cellular function. Here, we provide evidence that subcellular localization of POSH is regulated by a particular domain of the protein and POSH was colocalized with hepatocyte-growth-factor-regulated tyrosine kinase substrate (Hrs) on early endosomes via interaction of Hrs with POSH's two rear SH3 domains. Moreover, the RING domain of POSH specifically regulates the stability of Hrs, but not of JNK1, via a ubiquitin–proteasomal degradation pathway. Finally, we demonstrate that JNK1 does not interact with Hrs under the conditions of POSH interacted with Hrs, but instead reduces the POSH-catalyzed ubiquitination of Hrs and their reciprocal interaction. Together, these data suggest that POSH has a distinct role as a specific E3 ubiquitin ligase for Hrs on early endosomes, and there exists a relationship between its separate activities as a scaffold and as an E3.

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The role of LPA signaling in development of the anterior nervous system

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We have been interested in the role of the signaling phospholipid lysophosphatidic acid (LPA) and its receptors in regulation of cell behaviors during development. The phospholipid LPA signals through G-protein-coupled receptors to

influence cell morphology, gene expression, cell survival, and cell proliferation. Determining the role of LPA signaling within the nervous system has been difficult in mammalian models due to a significant amount of redundancy. We have begun to determine the role of the LPA receptor XLPA1 using *Xenopus laevis* as a model since there is less redundancy in this system. XLPA1 is expressed in the developing CNS throughout neurulation and later at tailbud stages in *Xenopus*. Loss of XLPA1 function, using morpholino oligonucleotides targeted to a specific blastomere fated to give rise to the nervous system, results in a loss of anterior neural structures including eye and neural tube within the forebrain. These structures can be rescued by reintroduction of a morpholino-resistant mRNA for XLPA1 into the same cell as the morpholino. In the loss of function embryos, this phenotype is due to increased levels of apoptosis and reduced levels of proliferation within the developing CNS. Expression of the neural induction and specification markers Xsox2 and Xpax6 is expressed in a normal pattern during early neural induction, but at later stages the expression is lost in an anterolateral domain of the CNS. These data suggest that XLPA1 signaling plays a central role in the CNS and is necessary as a survival signal.

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Signaling complexes regulating axon outgrowth

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Plenty of Src homology 3 domains (POSH) is a scaffold protein and ubiquitin ligase that interacts with multiple serine/threonine kinases and the small GTPase Rac. POSH is expressed in the developing central nervous system as well as in non-neural embryonic tissues, including lung, kidney and testis. We find that inhibition of POSH expression by RNAi in either mouse P19 neurons or mouse primary cortical neurons leads to enhanced axon outgrowth, while overexpression of POSH reduces process outgrowth. These observations suggest that POSH negatively regulates axon outgrowth. POSH is present in growth cones, suggesting that POSH may act in this compartment to regulate axon outgrowth. POSH interacts with Shroom, an F-actin binding protein, and RNAi-mediated inhibition of Shroom also leads to a dramatic increase in axon length. In addition, overexpression of the Shroom actin binding domain, a dominant negative protein, leads to enhanced axon outgrowth, suggesting that Shroom couples to the cytoskeleton to regulate process outgrowth. Taken together, our results suggest that the POSH–Shroom complex negatively regulates axon outgrowth. We propose that the POSH–Shroom complex impedes axon outgrowth by regulating the neuronal cytoskeleton. Supported by NIMH (ABV) and NINDS (DLT).

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Polycistronic RNA polymerase II expression vectors for RNA interference based on miR-155

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We have developed new RNA polymerase II expression vectors for RNA interference (RNAi), designated SIBR vectors, based upon miR-155 precursor in the mouse non-coding RNA BIC. At least two different synthetic miRNAs can be expressed from a single transcript without compromising inhibitory efficacy, allowing effective inhibition of two genes using a single vector. Alternately, multimerization of a single miRNA cassette up to at least eight copies can be used to increase the inhibition of a target mRNA. In addition, the miRNA can be located within an intron for coupled expression of the miRNA and a marker protein from a single transcript, facilitating the identification or selection of cells expressing the miRNA. Alternately, expression of a biologically active protein can be combined with RNAi for functional analyses of a downstream pathway. We show that intronic expression of two tandem SIBR cassettes each targeting one of two related kinases, B-Raf and c-Raf, provided effective knockdown of both endogenous proteins but did not reduce the level of the untargeted kinase ERK. Expression of an intronic SIBR cassette from a dual-intron vector further enhances inhibition, and this design can be combined with multimerization of the miRNA cassettes. The SIBR vectors offer a flexible system for RNAi, amenable for the analysis of combinations of genes with potentially redundant functions in developmental processes.

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Simple sequence domain of Ci regulates proteolytic processing

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One of the ways by which Hedgehog (Hh) signaling regulates the activity of the transcription factors Ci/Gli3 is by the proteolytic processing of the full-length protein into a truncated transcriptional repressor. We show that partial proteolysis is carried out by the proteasome and that two features of the Ci protein are required for processing. The first is the tightly folded zinc finger domain, and the second is a simple sequence domain positioned at an appropriate distance from the zinc fingers. The complexity of the simple sequence domain determines the extent of proteolytic processing. In tissue culture, substituting the Ci simple sequence domain with a lower complexity sequence gives rise to more repressor and greater repression of Hh target genes. In vivo, substituting a Ci protein with a lower complexity simple sequence domain for the endogenous Ci protein changes